

Leaf Water Balance During Oscillation of Stomatal Aperture

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Abstract. Continuous transpiration and β -gauge measurements were made on cotton plants, the stomatal apertures of which had been induced to oscillate, thus allowing a water balance to be made, and leaf potential to be measured as functions of time.

Analyses showed phase differences between the water entering and leaving the leaf. Also, from the phase relationship between the flow into the leaf and the water potential in the leaf it was shown that the water potential in the xylem of the plant also oscillated. This is proposed as a necessary condition for the stomates of all the leaves of a plant to oscillate in phase.

It is convenient to describe the dynamics of the phenomena using a simple electrical analogue, and the usefulness and limitations of the model are discussed.

Cyclic oscillations in stomatal aperture, transpiration rate and photosynthetic rate with periods of the order of 1 hr have been observed occasionally during the last 40 years. No one has yet elucidated the mechanism responsible for these oscillations although there is agreement that all these phenomena involve oscillations in stomatal aperture. For a particular plant, the amplitude of oscillation may vary with environmental conditions (2, 7, 14, 17) while the period remains the same. Yet the oscillations must be dependent on some relatively conservative structural feature, since many species exhibit more or less the same frequency of oscillation; most commonly the period is 20 to 50 min. There can, however, be considerable variation within a species. In this laboratory, cotton plants of the same variety grown in glasshouses in the summer months generally had a period of oscillation of about 40 min, but the period in the winter was often about an hour. Thus, some conservative property, which is none-the-less influenced by environment, is involved in these oscillations.

Furthermore, a restricted supply of water to the leaves is a necessary condition for the occurrence of the phenomenon (1, 3, 5, 12).

Oscillations in plant water loss, caused by periodic opening and closing of stomates, is associated with cyclic variations in leaf water status (1, 4). Estimates of leaf water status made with a β -gauge show that leaf water content is out of phase from its normal relationship with stomatal aperture, i.e., stomates are open when leaf water content is low and closed when the leaf is more turgid (4). Similarly, measurements of the water potential of leaves near the one being monitored for water loss indicate that leaf water potential is cycling (1). Changes in leaf water potential give rise to changes in the driv-

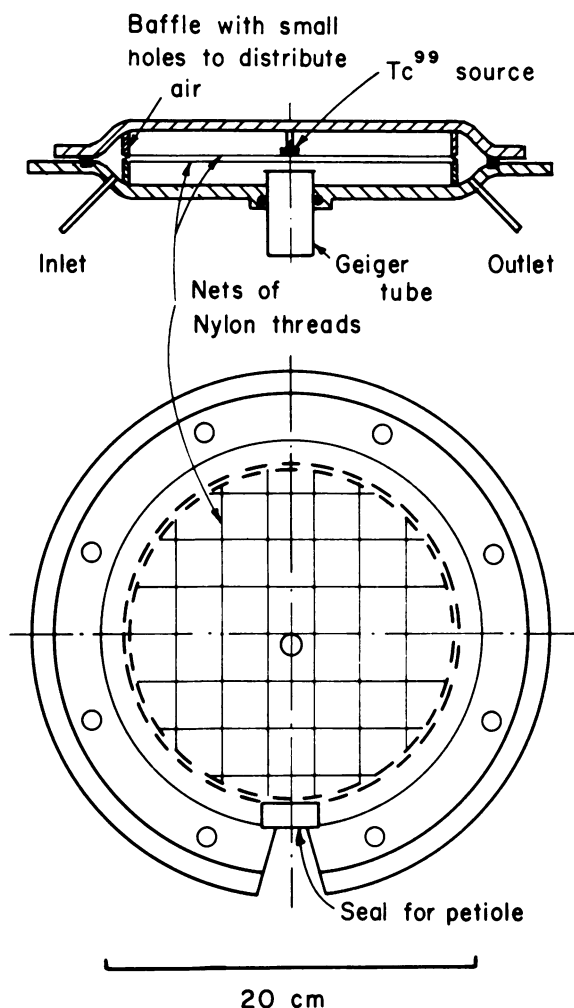
estimates of the superficial density (mg dm^{-2}) of leaf surface. Thus it is possible that, when a plant undergoes oscillations in stomatal aperture, there is cycling not only in the rate of water loss from the leaves but also in both the rate of water influx through the petiole and the water content of the leaf itself. This work was undertaken to show the relationship between the flow of water into the petiole and the rate of water loss to the atmosphere for a plant which has been induced to cycle, and to try to indicate the mechanism by which the oscillations are sustained.

Materials and Methods

Cotton plants (*Gossypium barbadense* L. var. Pima S-2) were grown in Hoagland's solution in a glasshouse and were about 2 months old when used. Cotton was chosen for this study because it cycles readily and has leaves with large interveinal areas for β -gauging.

A Plexiglas leaf chamber (Fig. 1), which had a β -gauge built into it, was used to enclose a leaf. The β -gauge (11) was made using a Tc-99 source and a Geiger-Muller tube, and was calibrated with aluminum absorbers. This β -gauge gave successive estimates of the enclosed leaf every 80 sec. Thus the rate of change of leaf water content on a unit area basis, \dot{m}_2 ($\text{mg dm}^{-2} \text{ min}^{-1}$) could be calculated by taking the derivative with time of the superficial densities. The derivatives at each time, t was obtained from the slope of the least-squares line passing through the 9 points bracketing the time, t .

The rate of water loss from the enclosed leaf, \dot{m}_3 ($\text{mg dm}^{-2} \text{ min}^{-1}$) was measured with a differential psychrometer (1, 13). Flow rates into and out of the leaf chamber, measured with rotameters and


 FIG. 1. Leaf chamber with β -gauge.

corrected for temperature and pressure, were averaged to give the flow rate used in calculations.

The rate of water flow into the leaf (\dot{m}_1) at each point in time was calculated from the rate of change of water content (\dot{m}_2) and the rate of water loss (\dot{m}_3) by the following formula:

$$\dot{m}_1 = \dot{m}_2 + \dot{m}_3$$

in the present experiments, the value of \dot{m}_1 and \dot{m}_3 are always positive, but \dot{m}_2 is positive or negative depending on whether the leaf has a net gain or loss of water.

A method was devised by which estimates of leaf water potential could be made from the superficial densities as measured with the β -gauge. At the end of every experiment, the final β -gauge reading was taken and immediately thereafter interveinal leaf discs were sampled for determination of relative turgidity (RT) from the formula $100 \times (FW - DW)/(TW - DW)$, where FW, DW, and TW are the fresh, dry and turgid weights of the tissue, respectively. In preliminary experiments, the rela-

tionship had been established between leaf water potential (ψ) as determined with a pressure chamber (8), and relative turgidity. For water potentials greater than -1400 J kg^{-1} the relationship may be expressed as:

$$\Psi = -(112 \pm 7) (100 - RT) (\text{J kg}^{-1}) \quad (\text{I})$$

where the limit is the sample standard deviation of the regression coefficient. If we define a constant, K, such that

$$FW = K\sigma \quad (\text{II})$$

where σ is the superficial density (mg dm^{-2}) as measured by β -gauging and FW is the fresh weight of the leaf in milligrams, then, by substitution in equation (I)

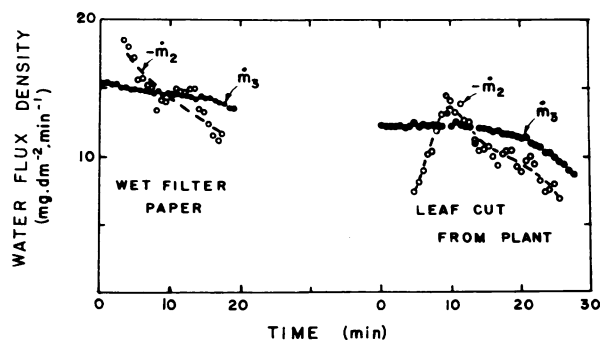
$$\psi = -11200 \frac{(TW - K\sigma)}{(TW - DW)} \quad (\text{III})$$

Equation (III) assumes that the leaf area does not change with water content. There would be no error from this assumption at the water potential at which K was determined, but based upon the data of Gardner and Ehlig (6) the error would be about 25 J kg^{-1} for each 100 J kg^{-1} departure from this potential. This precision was sufficient for the present work, in which we were interested mainly in phase differences.

Thus, by following changes in superficial density during an experiment, many successive estimates of leaf water potential could be made.

The apparatus was first tested with 2 simple experiments. First, wet filter paper was enclosed in the Plexiglas chamber so that \dot{m}_3 could be compared to \dot{m}_2 when \dot{m}_1 was zero. Fig. 2 shows that, while there are discrepancies between the 2 measurements, the mean transpiration rate calculated from β -gauge measurements agrees with the results obtained with the differential psychrometer.

In the second set of experiments, an attached leaf was allowed to come to a steady state in the chamber and the petiole was then cut in air so that \dot{m}_1 would be zero while \dot{m}_2 and \dot{m}_3 were measured. Fig. 2 shows that there is a good correlation between the rate of water loss as determined by the 2 methods. The value obtained from the β -gauge was about


 FIG. 2. Water loss from wet filter paper and a detached leaf by differential psychrometry, \dot{m}_3 , and from the superficial density, $-\dot{m}_2$.

20 % lower than the value obtained with the differential psychrometer. This discrepancy was ascribed mainly to the fact that the β -gauge measures the water in the interveinal leaf areas and the differential psychrometer measures the loss of water from the entire leaf. The veins hold a considerable volume

of water which moves into the interveinal areas so that, in this experiment, \dot{m}_1 for the interveinal areas is not actually zero.

Finally, the system was used on an attached leaf of a plant induced to cycle by imposing a single 20-min dark period.

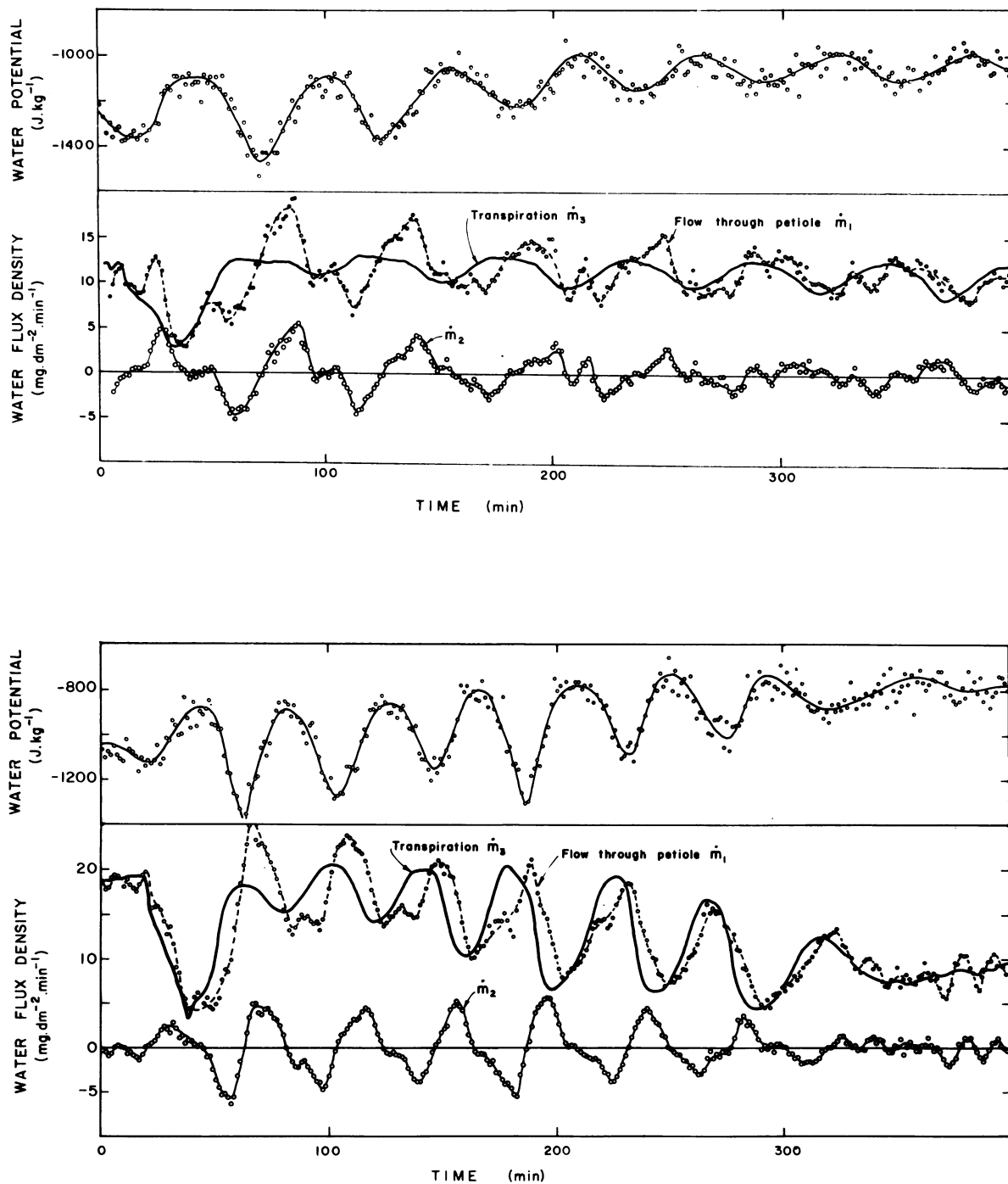


FIG. 3. Phase relationships between water potentials and fluxes of water per unit area of leaf, for oscillating leaves of cotton plants.

Results

The time courses of values of \dot{m}_1 , \dot{m}_2 , \dot{m}_3 and ψ for 2 of the experiments on an attached leaf of a cycling plant are presented in Fig. 3, A and B. All 4 parameters show oscillatory behavior with the same frequency; the oscillations are not true sine waves. For example, the oscillations in water potential show rounded peaks but sharp troughs, so that the curves are not symmetric about any horizontal line. The phase relationships between the various properties are similar in the 2 plants, but the amplitude in Fig. 3A is much smaller than that in 3B.

Discussion

It was deduced from the results that the water potential in the xylem oscillated, for otherwise the flow through the petiole (\dot{m}_1) would be exactly 180° out of phase with the oscillating water potential in the leaf.

Following in part Ehrler *et al.* (4), we suggest the following hypothesis for a leaf undergoing oscillations in stomatal aperture: at the end of a dark period, leaves are turgid but stomata are closed, so the system is displaced from its illuminated steady state. With light, the stomata open more or less synchronously and water loss occurs from the inner evaporation surfaces, *i.e.* the spongy mesophyll. The stomata open wider than would be normal for a leaf in a steady state; the rate of loss exceeds the rate of uptake so that the leaf water potential drops. There is a resistance to the movement of water from the guard cells to the cells where evaporation occurs so that there is a time lag in the response of the guard cells to the loss of turgor in the mesophyll (16). Therefore, before the stomata close, the mesophyll develops a water potential lower than the steady state value in the absence of oscillations. It is this low potential which then causes a rapid influx of water into the leaf to restore its turgor. Again there is a time lag in the response of the guard cells which open as they become more turgid, and the sequence of events repeats.

This hypothesis and the structure of the plant suggest an electrical analogue such as shown in Fig. 4, where control of the water content of the leaf

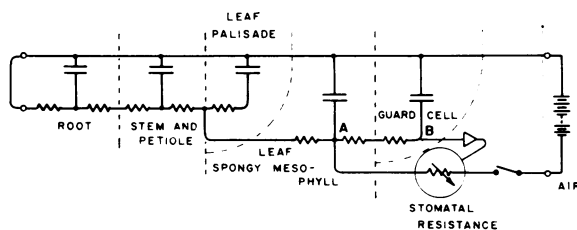


FIG. 4. Simplified electrical analog of a plant with roots in medium of zero potential. Open triangle signifies coupling between potential in guard cell and stomatal resistance.

depends upon the stomatal resistance. However, the stomatal aperture is controlled by the water potential in the leaf, so that the leaf water balance results from a control system with negative feedback. The circuit shown is related to that of a feedback oscillator (10). For oscillations to be sustained, a phase shift of 180° is required between points A and B, which necessitates at least 3 well chosen R-C units; consequently, the circuit, as it stands should not oscillate, although a damped oscillation is possible. Thus this simple model does not fully describe the dynamic behavior of an oscillating plant. However, the model was analyzed with an analogue computer using a range of feasible values for the parameters. Based upon observations when damped oscillations occurred, the model is consistent, qualitatively, with the following experimental results: 1. The average potential of the leaf was out of phase with the transpiration rate, which has also been reported previously (1,4). 2. The flow into the petiole was out of phase with the flow out of the leaf. 3. The water potential in the xylem oscillated.

A realistic model must also explain how root resistance (1) was implicated in the oscillation. For the model to sustain an oscillation there are 2 requirements: first the 180° phase shift already mentioned, and secondly the effective gain in the feedback loop must exceed the attenuation (or damping) of the water potential wave as it passes along the spongy mesophyll to the guard cell. In the present context the gain in the feedback loop is directly related to the slope of the curve of the stomatal conductance as a function of the steady state water potential in the leaf. This slope (15, 18) increases with decreasing water potential. It is suggested, therefore, that the necessary condition of restricting the supply of water to the leaves before oscillations will occur results from the need to have the water potential of the leaves at a value where the slope of the stomatal conductance *versus* potential is sufficiently great.

In passing it is noted that the model proposed by Meleschenko and Karmanov (9) oscillates, but has the serious disadvantage that arrangement of components do not show direct physical correspondence with a plant. The present model would probably oscillate if the lumped R-C between A and B of Fig. 4 were partitioned into sufficient separate R-C units, consistent with the fine structure of the cells in the flow path. At present it is not clear how the partitioning should be done.

All of the leaves on a plant cycle in phase with one another (3). This has also been observed in our laboratory in time-lapse motion pictures which show that, as leaves alternately wilt and become turgid, they are in phase. The suggestion that the xylem potential cycles, and acts as a "forcer" to bring neighboring leaves into synchronous oscillation with one another (9), is supported by the conclusion from the present work that the water

potential in the xylem oscillated. Oscillation of the xylem potential is proposed as a necessary condition for all the leaves to oscillate in phase.

If stomata can oscillate in some plants, then why don't they oscillate all the time? Stalfelt (14) has reported that stomata on a leaf surface are in continuous oscillation, but are not necessarily in phase with one another. Probably the treatments used to initiate these oscillations synchronize stomatal activity and make the normal oscillations apparent. At the end of a dark period, all stomata are more or less closed, and open synchronously with light. The plant is then put into a dynamic situation where stomata may continue to oscillate synchronously if they are coupled to one another and if the resistances and capacitances in the system allow cycling.

Plants with these cyclic oscillations can provide insight into the mechanisms of water movement from one part of a plant to another. The methods devised in this work provide tools for gaining some of this insight. The simultaneous measurement of water status and water loss of an attached leaf has given information on influx into the leaf and has led to inferences about xylem water potential. The ability to assess the water potential of interveinal leaf areas continuously should lead to further studies in the dynamic relationships of water in leaves.

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